are representative of the earliest land plants, the transition from an unbranched to a branched form was one of the most significant steps in land plant evolution, paving the way for indeterminate shoot growth in the sporophyte. The *P. patens pinB* mutant phenotype suggests that this transition may have been facilitated by altering polar auxin transport processes in the sporophyte generation. An alternative view, based on the discovery of branched fossils that predate vascular plants, suggests that the earliest land plants were branched and that extant bryophytes lost branching function [19]. Either way, the modification of PIN-mediated polar auxin transport can now be proposed as a major driver of morphological novelty during land plant evolution.

References

1. Went, F.W. (1926). On growth accelerating substances in the coleoptile of *Avena sativa*. Proc. Kon. Ned. Akad. Wet. *30*, 10–19.
2. Rubery, P.H., and Sheldrake, A.R. (1974). Carrier-mediated auxin transport. Planta *118*, 101–121.
3. Galweiler, L., Guan, C., Muller, A., Wisman, E., Mendgen, K., Yephremov, A., and Palme, K. (1998). Regulation of polar auxin transport by AtPIN1 in Arabidopsis vascular tissue. Science *282*, 2226–2230.
4. Krecek, P., Skupa, P., Libus, J., Naramoto, S., Tejos, R., Friml, J., and Zazimalova, E. (2009). The PIN-FORMED (PIN) protein family of auxin transporters. Genome Biol. *10*, 249.
5. Bennett, T., Brockington, S.F., Rothfels, C., Graham, S.W., Stevenson, D., Kutchan, T., Rolf, M., Thomas, P., Wong, G.K., Leyser, O., *et al*. (2014). Paralogous radiations of PIN proteins with multiple origins of noncanonical PIN structure. Mol. Biol. Evol. *31*, 2042–2060.
6. Fujita, T., Sakaguchi, H., Hiwatashi, Y., Wagstaff, S.J., Ito, M., Deguchi, H., Sato, T., and Hasebe, M. (2008). Convergent evolution of shoots in land plants: lack of auxin polar transport in moss shoots. Evol. Dev. *10*, 176–186.
7. Ruhfel, B.R., Gitzendanner, M.A., Soltis, P.S., Soltis, D.E., and Burleigh, J.G. (2014). From algae to angiosperms-inferring the phylogeny of green plants (Viridiplantae) from 360 plastid genomes. BMC Evol. Biol. *14*, 23.
8. Graham, L.E.C., Cook, M.E., and Busse, J.S. (2000). The origin of plants: body plan changes contributingto a major evolutionary radiation. Proc. Natl. Acad. Sci. USA *97*, 4535–4540.
9. Reinhardt, D., Pesce, E.R., Stieger, P., Mandel, T., Baltensperger, K., Bennett, M., Traas, J., Friml, J., and Kuhlemeier, C. (2003). Regulation of phyllotaxis by polar auxin transport. Nature *426*, 255–260.
10. Smith, R.S., Guyomarc'h, S., Mandel, T., Reinhardt, D., Kuhlemeier, C., and Prusinkiewicz, P. (2006). A plausible model of phyllotaxis. Proc. Natl. Acad. Sci. USA *103*, 1301–1306.
11. Ashton, N.W., Grimsley, N.H., and Cove, D.J. (1979). Analysis of gametophytic development in the moss, *Physcomitrella patens*, using auxin and cytokinin resistant mutants. Planta *144*, 427–435.
12. Bennett, T.A., Liu, M.M., Aoyama, T., Bierfreund, N.M., Braun, M., Coudert, Y., Dennis, R.J., O'Connor, D., Wang, X.Y., White, C.D., *et al*. (2014). Plasma membrane-targeted PIN proteins drive shoot development in a moss. Curr. Biol. *24*, 2776–2785.
13. Viaene, T., Landberg, K., Thelander, M., Medvecka, E., Pederson, E., Feraru, E., Cooper, E.D., Karimi, M., Delwiche, C.F., Ljung, K., *et al*. (2014). Directional auxin transport mechanisms in early diverging land plants. Curr. Biol. *24*, 2786–2791.
14. Reutter, K., Atzorn, R., Hadeler, B., Schmulling, T., and Reski, R. (1998). Expression of the bacterial *ipt* gene in *Physcomitrella* rescues mutations in budding and in plastid division. Planta *206*, 196–203.
15. Chapman, E.J., and Estelle, M. (2009). Mechanism of auxin-regulated gene expression in plants. Annu. Rev. Genet. *43*, 265–285.
16. Haga, K., and Sakai, T. (2012). PIN auxin efflux carriers are necessary for pulse-induced but not continuous light-induced phototropism in Arabidopsis. Plant Physiol. *160*, 763–776.
17. Friml, J., Wisniewska, J., Benkova, E., Mendgen, K., and Palme, K. (2002). Lateral relocation of auxin efflux regulator PIN3 mediates tropism in Arabidopsis. Nature *415*, 806–809.
18. Ding, Z., Galvan-Ampudia, C.S., Demarsy, E., Langowski, L., Kleine-Vehn, J., Fan, Y., Morita, M.T., Tasaka, M., Fankhauser, C., Offringa, R., and Friml, J. (2011). Light-mediated polarization of the PIN3 auxin tranporter for the phototropic response in Arabidopsis. Nat. Cell Biol. *13*, 447–452.
19. Edwards, D., Morris, J.L., Richardson, J.B., and Kenrick, P. (2014). Cryptospores and cryptophytes reveal hidden diversity in early land floras. New Phytol. *202*, 50–78.

Department of Plant Sciences, University of Oxford, South Parks Rd, Oxford OX1 3RB, UK.
E-mail: jane.langdale@plants.ox.ac.uk

http://dx.doi.org/10.1016/j.cub.2014.10.047

Animal Evolution: Stiff or Squishy Notochord Origins?

The notochord is considered an evolutionary novelty and one of the defining characters of chordates. A new study of an annelid challenges this view and proposes an earlier evolutionary origin in the most recent common ancestor of chordates and annelids.

Andreas Hejnol[1,2] and Christopher J. Lowe[1,2]

Chordates (sea squirts, lancelets and vertebrates) are one of the animal groups with the most complex body plans. One of the defining characters of this group is a rod-like elastic structure on the dorsal side of their body that is commonly known as the 'notochord' or *chorda dorsalis* [1]. This structure stiffens the body and functions also as an attachment site for lateral muscle packages, called 'somites', which helps with undulating swimming movements. The notochord develops in the embryo from a dorsal mesodermal population of cells that form a rod. In some lineages (ascidian larvae, hagfish, coelacanth), these cells become vacuolarized, while in others (*Branchiostoma*) they become muscular. The notochord has also an important developmental signaling function, for instance as a source of BMP antagonists during the formation of the overlying neural plate and as the initial source of the signaling molecule Sonic Hedgehog (Shh) to ventralize the forming nerve cord [2]. In most craniates (bony fish, birds, mammals), however, the notochord is a transient structure that disappears after it has accomplished its signaling function and is replaced by the backbone composed of vertebrae made out of cartilage or bones.

What is the evolutionary origin of this defining chordate character? Are there any comparable structures in more closely related deuterostome lineages that might hint to its origin or can it be that it is an evolutionary novelty (Figure 1)? The closest group to chordates are the Ambulacraria, comprising hemichordates (acorn worms) and echinoderms (sea urchins, sea stars and sea cucumbers) and perhaps *Xenoturbella* [3]). As there are no strong contenders for notochord-like structures in these animals, the notochord is generally considered an evolutionary innovation of chordates [4,5]. However, a recent paper by Lauri and coauthors [6] challenges this widely held view.

The authors [6] searched for cells that resemble the notochord in the polychaete worm *Platynereis dumerilii*, a member of the distantly related

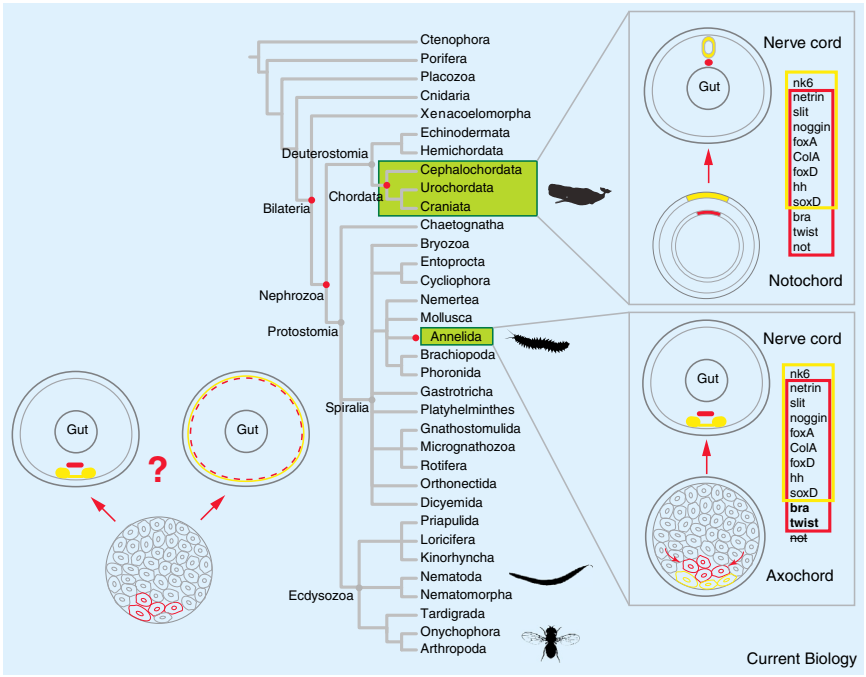

Figure 1. Homology or homoplasy in the evolution of the notochord?

Animal phylogeny (after [3]) illustrating the position of chordates and annelids (green rectangles). Pictograms for the model systems *Caenorhabditis elegans* and *Drosophila melanogaster* are added as points of orientation. A comparison of the development of the notochord and 'axochord' is illustrated on the right. The 'molecular fingerprint' is illustrated by the list of genes that are co-expressed. Yellow rectangles surround the 'fingerprint-genes' of the neural tissue while the red rectangles are surrounding the genes of the notochord or axochord precursors. Two alternative hypotheses about the ancestral state for Bilateria are shown left of the phylogeny. Left: hypothesis according to Lauri *et al*. [6] indicates the formation of a ventral median muscle with stiff architecture (red) formed by ventral mesodermal precursurs (red) placed adjacent to a centralized ventral nerve cord. Right: the precursors, if present, can give rise to mesodermal tissues (muscle sheath or parenchyme) that later diversify into different mesodermal structures in animal lineages.

protostome group Annelida. They conducted a thorough investigation of the development of the annelid using light-sheet microscopy and molecular markers to search for cells that share the following features: they are of mesodermal origin, as the notochord in chordates develops from the mesodermal germ layer; they are specified by a set of genes similar to the one that defines the notochord in chordates; and finally, candidate cells should be present on the ventral side of the annelid and not on the dorsal side, as in chordates. This position is expected according to the 'dorsal-ventral-inversion' hypothesis, which posits that the ventral side of non-chordates corresponds to the dorsal side of chordates [7] based on the inversion in the orientation of the molecular BMP2/4–Chordin axis that defines the dorsoventral axis in chordates and other bilaterians. In their survey, the authors indeed found ventral, mesodermal cells during the early development of the annelid that express a specific combination of transcription factors and signaling molecules that are orthologues of genes known to play key roles during chordate notochord development (Figure 1).

As functional studies by gene knockdown are difficult at late embryonic stages, the approach largely relies on comparing the co-expression of genes, or a so-called 'molecular fingerprint' [8]. The rationale behind this approach is that homologous cell types in different species express a combination of transcription factors, signaling molecules and structural genes that can be highly similar even in distantly related groups. The potential problem with this approach is that conserved 'molecular fingerprints' can be expressed in similar combinations in structures that are clearly not homologous, so convergence cannot be excluded.

What do these mesodermal cells become in the annelid when there is no notochord? Lauri *et al.* [6] show that these cells form a median muscle to which lateral muscles are attached. This muscle is located between the ventrally centralized nervous system and the digestive tract, and based on its relative spatial position to the notochord, the authors call this muscle an 'axochord'. The authors conclude from the similarities in the combination of expressed genes that these median mesodermal cells share a common evolutionary origin with the cells that make up the chordate notochord. These cells must thus have been present in the last common ancestor of the annelid worm and chordates.

If this is right, which anatomical structure did this population of cells give rise to in their last common ancestor? Lauri *et al.* [6] are cautious on this point, but suggest that they formed some kind of ventral musculature (Figure 1). In fact, ventral musculature is present in most bilaterian animals and has been considered to be part of their unifying traits [3,9]. The organization of ventral musculature, however, is very varied across taxa and is adapted to the specific locomotion demands of each group. In animals that glide on cilia (xenacoelomorphs, platyhelminthes, nemerteans and others), it is part of an orthogon-like muscular mesh surrounding the body. Other animals (priapulids, nematodes and nematomorphs) have a tight muscular sheath that surrounds the whole body. Some animal groups show more discrete longitudinal strands of median and paired longitudinal muscle similarly organized as in the annelids, which have evolved undulating locomotion with unique appendages called 'parapodia'.

How do the findings of Lauri *et al.* [6] and their hypothesis relate to the origins of the notochord? Did the notochord evolve from ventral musculature of an ancestor? When we embrace a comparative approach that considers the vast diversity in bilaterian dorsoventral organization of mesoderm, the ability to precisely reconstruct the morphological details of an ancestral ventral midline, based on developmental studies that sample a mere fraction of that diversity, is debatable. The observed similarity in gene expression between annelids and chordates leaves us with a large knowledge gap about the lineages of

animals that are positioned between annelids and chordates on the evolutionary tree (Figure 1), particularly groups with a contrasting organization of ventral musculature. These missing data need to be generated before we can be sure if this similarity is based on shared ancestry or convergence.

Only when similar mesodermal precursor cells are revealed at the ventral midline of animal groups that bridge the evolutionary distance between annelids and chordates or show different ventral mesodermal architectures (such as hemichordates, nemerteans, brachiopods, priapulids, flatworms) can we more confidently reconstruct that character in their stem species. Furthermore it remains unclear whether and how the genes expressed in the ventral mesodermal cells interact in annelids to specify and pattern the resulting structure. The data so far are only based on the combined expression of genes and not their functional interactions. Such functional studies would be critical, as only two transcription factors, *brachyury* and *twist*, are separating the combinatorial fingerprint of the mesodermal candidate cells from the neighboring cells of nerve cord in *Platynereis* (Figure 1).

Homology implies a common evolutionary origin and can only be discriminated from homoplasy — similar structures that do not share a common evolutionary ancestry — on the basis of a solid phylogenetic framework [3]. This comparative approach can tell us whether a common structure is evolutionarily conserved or whether it is convergent. It thus can help us to escape from being tricked into asserting common origin by superficial similarities. Although Lauri *et al.* [6] suggest a possible homology of the annelid 'axochord' and the chordate notochord, these two structures are not likely to be homologs. Many similarities the authors describe — such as similar developmental origin, position in the animal between nerve cord and blood vascular system and the function as stiffening tissue to which lateral muscles are attached — are probably homoplasies, as annelids and chordates are separated by multiple lineages that appear to lack such elaborated structures. The combinatorial gene expression in the cells that give rise to both structures may well play an early role in the specification of the ventral mesodermal precursors. However, the resulting structures (notochord, axochord) are a further developmental elaboration of these precursor cells and may be regulated by contrasting downstream effector genes, suggesting that the structures may have evolved convergently.

The significance of this debate should not be underestimated: if early bilaterians were small, simple animals with modest body plans, then subsequent bilaterian evolution was characterized by innovation, novelty and independent convergent evolution of complex body plans in several lineages [10]. However, if they were complex, as implied by homology of axochord and notochord or a complex central nervous system, then bilaterian evolution is defined by pervasive loss of morphological complexity in the majority of bilaterian lineages and conservation and modification in a few.

References

1. Stemple, D.L. (2004). The notochord. Curr. Biol. *14*, R873–R874.
2. Stemple, D.L. (2005). Structure and function of the notochord: an essential organ for chordate development. Development *132*, 2503–2512.
3. Dunn, C.W., Giribet, G., Edgecombe, G.D., and Hejnol, A. (2014). Animal phylogeny and its evolutionary implications. Annu. Rev. Ecol. Evol. Syst. *45*, 371–395.
4. Satoh, N., Tagawa, K., Lowe, C., Yu, J.-K., Kawashima, T., Takanashi, H., Ogasawara, M., Kirschner, M., Hisata, K., Su, Y.-H., *et al*. (2014). On a possible evolutionary link of the stomochord of hemichordates to pharyngeal organs of chordates. Genesis, http://dx.doi.org/10.1002/dvg.22831.
5. Satoh, N., Tagawa, K., and Takahashi, H. (2012). How was the notochord born? Evol. Dev. *14*, 56–75.
6. Lauri, A., Brunet, T., Handberg-Thorsager, M., Fischer, A.H.L., Simakov, O., Steinmetz, P.R.H., Tomer, R., Keller, P.J., and Arendt, D. (2014). Development of the annelid axochord: insights into notochord evolution. Science *345*, 1365–1368.
7. Arendt, D., and Nübler-Jung, K. (1994). Inversion of dorsoventral axis? Nature *371*, 26.
8. Arendt, D. (2008). The evolution of cell types in animals: emerging principles from molecular studies. Nat. Rev. Genet. *9*, 868–882.
9. Ax, P. (1996). Multicellular Animals, Volume I (Berlin: Springer Verlag).
10. Hejnol, A., and Martindale, M.Q. (2008). Acoel development supports a simple planula-like urbilaterian. Philos. Trans. R. Soc. Lon. B *363*, 1493–1501.

[1]Sars International Centre of Marine Molecular Biology, University of Bergen, Norway. [2]Hopkins Marine Station, Department of Biology, Stanford University, USA.
E-mail: andreas.hejnol@sars.uib.no, clowe@stanford.edu

http://dx.doi.org/10.1016/j.cub.2014.10.059

Climate Change: Bees and Orchids Lose Touch

Spring temperature increases could differentially affect flowering times and pollinator flight periods, leading to asynchrony and reduced pollination. A specialist orchid–bee study combining herbarium, museum and field data shows that bee flight dates are advancing faster than orchid flowering, which could lead to significant future uncoupling.

Pat Willmer

Back in the 1970s and 80s, when we first began to wake up to the causes and predicted consequences of global warming, some biologists were already concerned that a warming world would gradually provoke the uncoupling of important species interactions. An upset to the delicate balances between partner organisms involved in mutualisms such as pollination or seed dispersal could occur in earlier and/or warmer springs through differential thermal effects on mobile animals (able at least in principle to move away from areas where the climate became unsuitable) and on sessile plants (whose ability to 'migrate' may operate on a much longer timescale). In turn this could potentially destabilise ecosystems and have severe knock-on effects for human food security. However, nailing down some real evidence for this particular climate change outcome has proved tricky, not